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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/607,903	06/27/2003	Gjalt W. Huisman	MBX 025 DIV CON	7536

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EXAMINER	
HUTSON, RICHARD G	
ART UNIT	**PAPER NUMBER**
1652	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/607,903	HUISMAN ET AL.	
	Examiner	Art Unit	
	Richard G. Hutson	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *9/22/2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3,4,6,7,11,12,14-16,19* is/are pending in the application.

4a) Of the above claim(s) *11,12,14-16 and 19* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3,4,6 and 7* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicant's cancellation of claim 21, amendment of claim 1, in the paper of 9/22/2010, is acknowledged. Claims 1, 3, 4, 6, 7, 11-12, 14-16 and 19 are still at issue and are present for examination.

Applicants' arguments filed on 9/22/2010, have been fully considered and are deemed to be persuasive to overcome some of the rejections previously applied. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn.

Claims 11, 12, 14-16 and 19 are withdrawn from further consideration by the examiner, 37 CFR 1.142(b), as being drawn to a non-elected invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 3, 4, 6 and 7, are rejected under 35 U.S.C. 103(a) as being unpatentable over Greer et al. (WO 94/10289 (1994)), Atkinson et al. (Biochemical Engineering and Biotechnology Handbook 2nd edition, Stockton Press: New York, 1991) and Lee et al. (Production of poly(hydroxyalkanoic Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).acid, Adv.

Biochem. Eng. Biotechnol. 52:27-58, 1995), in view of Liebl et al. (J. Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).

A similar rejection was stated in the previous office action as it applied to previous claims 1, 3, 4, 6 and 7. In response to this rejection applicants have cancelled claim 21 and amended claim 1, and traverse the rejection as it applies to the claims in light of the filed declaration.

The rejection as it applies to applicants newly amended claims is repeated herein. Greer et al. teach that the degradation or removal of nucleic acids from cell lysates during fermentation is important because they form solutions of high viscosity which interfere with subsequent processing. Greer et al. specifically teach the usefulness of peroxide degradation in the recovery of intracellularly produced materials, in particular polyhydroxyalkanoate polymers, from bacterial cell lysates. Greer et al. further teach that nucleases can also be added to a cell lysate in order to degrade the nucleic acid although nucleases are expensive (page 1, lines 25-31).

Liebl et al. teach the expression, secretion and processing of *Staphylococcal aureus* nuclease by *Corynebacterium glutamicum*. Liebl et al. teach that Corynebacterium glutamicum is closely related to other “amino acid-producing corynebacteria” and these organisms are used for the industrial production of certain amino acids. Liebl et al. genetically engineer *Corynebacterium glutamicum* to express the *Staphylococcal aureus* nuclease. Liebl et al. teach that this *staphylococcal* nuclease is a heat-stable, excreted nuclease and a biochemically well characterized enzyme.

Miller et al. teach the secretion and processing of *Staphylococcal aureus* nuclease in *Bacillus subtilis*.

Atkinson et al. teach all aspects of biochemical engineering and biotechnology, including properties of microorganisms, microbial activity, product formation, fermentation processes, downstream processes and product recovery processes. Atkinson et al. also teach many products that can be produced biochemically such as antibiotics, organic acids, amino acids, proteins, vitamins, polyhydroxyalkanoates and polysaccharides. Atkinson et al. specifically teach many of the industrial production characteristics for a number of commercially important compounds, for example Atkinson et al. teach that *Alcaligenes eutrophus (Ralsotnia eutropha*) has been studied in detail due to its ability to accumulate large amounts of P(3HB) (i.e. ability to grow to cell densities of approximately 85 g/l and produce P(3HB) at 61.5 g/l, or 80% wt/wt of dry cell mass, page 30 through 32).

Lee et al. teach several processes developed for the production of various poly(hydroxyalkanoic acids) including various microorganisms used and the optimization of fermentation conditions.

One of ordinary skill in the art would have been motivated to genetically engineer a *Alcaligenes eutrophus (Ralsotnia eutropha*) polyhydroxyalkanoates producing bacterial strain, as taught by Atkinson et al., to express the *Staphylococcal aureus* nuclease as taught by Liebl et al. or Miller et al. or a homologous nuclease gene that has been modified to enhance nuclease activity, so that this bacterial strain would express a nuclease which is secreted into the periplasmic space in an amount effective

to degrade at least 95% of all the nucleic acid released following lysis of the cells in less than 24 hours and reduce the viscosity of a cell lysate in a bacterial cell culture having a density of at least 50 g/l so that recovery of product is enhanced. A nuclease excreted into the medium as a result of such a genetically engineered bacterial strain would a inherently result in the degradation of at least 95% of all the nucleic acid released following lysis of the cells in less than 24 hours. The motivation for producing a nuclease by a genetically engineered bacterial strain used in the fermentation process is to reduce the amount of nucleic acids in the medium which result in an increase in the viscosity of the medium, causing problems in the downstream processing steps, as taught by Greer et al. Greer et al. give further motivation for genetically engineering a bacterial strain to express a nuclease, because they teach that purified preparations of nucleases are expensive and a bacterial strain that was genetically engineered to express a nuclease activity would not require an external nuclease or hydrogen peroxide to be added to the fermentation. One would have had a reasonable expectation of success because both Liebl et al. and Miller et al. were able to express functional *Staphylococcal aureus* nuclease in different bacterial species, specifically *Corynebacterium glutamicum* and *Bacillus subtilis* and Liebl et al. teach that the *Staphylococcal aureus* nuclease is a heat-stable biochemically well characterized enzyme. One would have been further motivated to engineer the bacterial strain to secrete the nuclease into the growth medium in an effective amount to enhance the recovery of product from the growth medium. Alternatively one would have been motivated to engineer a homologous heterologous nuclease gene into the chromosome

of the bacterial host so that the nuclease activity can be increased for the same reasons as stated above for the introduction of the heterologous *Staphylococcal* nuclease.

Further, one would have been motivated to optimize the above fermentation conditions as taught by Lee et al. in order to more efficiently produce the desired product, polyhydroxyalkanoates as taught by Atkinson et al. Optimization of fermentation conditions includes the choice of the bacterial host such as *Alcaligenes eutrophus*, *Alcaligenes latus*, *Azotobacter vinelandii*, *Pseudomonas oleovorans*, *Pseudomonas resinovorans*, *Pseudomonas acidovorans* and *Escherichia coli* or any other microorganism which produces the desired product as taught by Atkinson et al. or Lee et al. For example, Atkinson et al. teach that *Alcaligenes eutrophus* (Ralstonia eutropha) has been studied in detail due to its ability to accumulate large amounts of P(3HB) (i.e. ability to grow to cell densities of approximately 85 g/l and produce P(3HB) at 61.5 g/l, or 80% wt/wt of dry cell mass, page 30 through 32). It would have been obvious to use a bacterial strain which grows to a high cell density and/or which produces a high level of the desired product.

Applicants Argument:

As in previous responses to similar rejections, applicants again review the legal standard, and then review applicant's interpretation of what each of the references teaches. After this analysis, applicants submit that "A combination of Greer, Liebl, Miller, Atkinson and Lee does not recite all of the elements of the claims". In response to this argument applicants are again reminded that this rejection is based upon the

obviousness of the claims in light of the teachings of the prior art references and that it is unnecessary for the combination of Greer, Liebl, Miller, Atkinson and Lee to recite all of the elements of the claims, in order for the claims to render obvious the rejected claims.

Applicants submit that Claim 1 as amended defines a bacterial strain producing polyhydroxyalkanoates, wherein the bacteria express a heterologous nuclease gene or a genetically modified homologous nuclease gene, the product of which is accumulated in the periplasmic space in an amount effective to degrade at least 95% of all of the nucleic acid released following lysis of cells in the growth medium in less than 24 hours so that recovery of the product is enhanced. Applicants characterization of claim 1 is appreciated, however, the reference to "a genetically modified homologous nuclease gene" is believed to be a mischaracterization of claim1, as currently amended in the amendment of 9/22/2010. Similarly, applicant's argument as it applies to homologous nucleases is disregarded, as the claims are currently drawn to those strains expressing a heterologous nuclease.

Applicants argument is on the basis that applicants submit that the Examiner has not identified any disclosure in any of the references cited which shows expression of nuclease in a gram negative bacteria having a periplasmic space so that the nuclease accumulates in the periplasmic space. Thus, the Examiner has not established a prima facie case of obviousness.

Applicants note the Examiner has previously asserted that one would have been motivated to engineer a bacterial strain to express Staphylococcal aureus nuclease as

taught by Liebl or Miller, or a homologous nuclease gene that has been modified to enhance nuclease activity, so that this bacterial strain would produce and excrete the nuclease into the bacterial growth medium as part of a fermentation process. Applicants note that the claims now require that the nuclease is secreted into the periplasmic space, not the growth medium and that the references alone or in combination do not disclose secretion of nuclease into the periplasmic space of gram negative bacteria and therefore do not meet all of the claimed limitations.

Applicants further argue that the combined references do not provide any expectation of success in arriving at the claimed process . Applicants support this line of reasoning on the basis that the Examiner's assertion, that one would have a reasonable expectation of success in arriving at the bacteria required by the claimed process because both Liebl and Miller were able to express nuclease in different bacterial species, does not provide any reasons why merely expressing nuclease in bacteria as disclosed in Liebl and Miller provides an expectation of success in arriving at bacteria exporting nuclease to the periplasmic space, since neither of Liebl or Miller is concerned with engineering bacteria to export nuclease into the periplasmic space. Applicants submit that release of nuclease into the culture medium is not release into the periplasmic space as required by the claims. Applicants further submit that when the nuclease is released into the cell culture medium, the bacteria inherently cannot meet the limitation that the nuclease is exported into the periplasmic space in an amount effective to degrade at least 95% of all of the nucleic acid released following lysis of

cells. Applicants submit that there is no nuclease in the periplamic space, when the nuclease is secreted into the culture medium. Therefore, neither of Liebl or Miller can provide an expectation of success that the claimed method would work.

In support of applicants argument that the references of Liebl and Miller do not provide an expectation of success that the claimed method would work, applicants point to applicants data on page 15, Table 1, in which applicants submit that out of 50 nuclease positive cells, only a very few showed high level of nuclease activity. Applicants submit that his is a critical feature of the process using the claimed bacterial strain, required for scale up for commercial production of polymer.

Finally, applicants submit that, as discussed above, the claimed process presents two advantages which are not contemplated or disclosed in the cited art; (i) exporting the nuclease into the periplasmic space sequesters the expressed nuclease until it is released when desired during the fermentation process, by cell lysis, and (ii) sequestering the nuclease in the periplasmic space protects the cells themselves from the toxic effects of nuclease, had it been secreted into the culture medium, allowing accumulation of significantly higher levels than can be achieved in gram positive cells. Applicants submit that the combination of references cited by the Examiner cannot provide a motivation to modify the disclosures therein in order to arrive at the claims or an expectation of success in arriving at the claimed process, where the prior references combined do not contemplate or disclose all of the claimed elements. For at least these reasons, the claims as amended are non- obvious over the combination of the prior art.

Applicants submit in summary, the prior art cited by the examiner fails to teach genetic engineering of an organism to export a nuclease into the periplasmic space. The evidence in the specification establishes that even when intentionally engineered, an intense selection process is required to identify organisms expressing sufficient nuclease to degrade the majority of nucleic acid released when the cells are lysed. This is not taught by the prior art.

Applicant's amendment of the claims and applicants complete argument is acknowledged and has been carefully considered, however, continues to be found non-persuasive for the reasons previously stated and for those reasons stated herein.

With regard to applicants submission that the Examiner has not identified any disclosure in any of the references cited which shows expression of nuclease in a gram negative bacteria having a periplasmic space so that the nuclease accumulates in the periplasmic space, applicants are again reminded that the current rejection is based upon the obviousness of the combination of references and as previously stated, one of ordinary skill in the art would have been motivated to genetically engineer an *Alcaligenes eutrophus (Ralsotnia eutropha*) polyhydroxyalkanoates producing bacterial strain, as taught by Atkinson et al., to express the *Staphylococcal aureus* nuclease as taught by Liebl et al. or Miller et al. so that this bacterial strain would express a nuclease which is secreted into the periplasmic space in an amount effective to degrade at least 95% of all the nucleic acid released following lysis of the cells in less than 24 hours and reduce the viscosity of a cell lysate in a bacterial cell culture having a density of at least 50 g/l so that recovery of product is enhanced.

It is acknowledged that Applicants note that the claims now require that the nuclease is secreted into the periplasmic space, not the growth medium and that the references alone or in combination do not disclose secretion of nuclease into the periplasmic space of gram negative bacteria and therefore do not meet all of the claimed limitations. While the references do not specifically disclose secretion of nuclease into the periplasmic space of gram negative bacteria, this limitation of applicants newly amended claims is considered to be an inherent property of the bacterial cells made obvious by Atkinson et al., which express the *Staphylococcal aureus* nuclease as taught by Liebl et al. or Miller et al. The inherency of the secretion of the nuclease in the obvious bacterial strain is based upon the reference of Boynton et al., Applied and Environmental Microbiology, Vol 65, No. 4, pp 1524-1529, April 1999, in which the authors of the reference teach the construction of nuclease integrants of PHA producing *R. eutropha* (*Alcaligenes eutropha*). It is recognized that the reference of Boynton et al. may not be available as prior art, however, this is not necessary since the reference is only being used to evidence those properties of the made obvious genetically engineered bacterial strain. These PHA producing *R. eutropha* cells were generated using the same nuclease encoding gene taught by Leibl et al. above (See Boynton et al. Materials AND Methods, p 1524 and Figure 3, p 1527 and supporting text p 1526). Boynton et al. teach that the transformant "R. eutropha secreted nuclease into the periplasm but not into the growth medium" (p1526, Construction of nuclease integrants of other PHA producers). Thus the bacterial strain that is obvious over the above references inherently produces the nuclease such that it is secreted into the

periplasmic space. Boynton et al. further characterize the nuclease activity of the periplasmic nuclease of the transformed *R. eutropha* cells in the data provided in Figure 3 and the supporting text, in which they show the result of chrosomal DNA treatment with periplasmic fractions of *R. eutropha* MBX917 (::nuc-kan) (lane 4) and *R. eutropha* NCIMB40124HD untransformed parent strain (lane 5) at 37°C for 1 hour. As is evident from the results of Figure 3, after only one hour of incubation with the transformed *R. eutropha* periplasmic fraction, all of the visible high molecular weight DNA was digested to smaller molecular weight fragments. Based upon this level of digestion of high molecular weight chromosomal DNA for only one hour at 37°C, clearly the level of nuclease produced in the transformed *R. eutropha* strain would digest at least 95% of all nucleic acid released following lysis of the bacterial cells in a 24 hour period.

Thus claims 1, 3, 4, 6 and 7, remain rejected under 35 U.S.C. 103(a) as being unpatentable over Greer et al. (WO 94/10289 (1994)), Atkinson et al. (Biochemical Engineering and Biotechnology Handbook 2nd edition, Stockton Press: New York, 1991) and Lee et al. (Production of poly(hydroxyalkanoic Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).acid, Adv. Biochem. Eng. Biotechnol. 52:27-58, 1995), in view of Liebl et al. (J. Bacteriology, 174(6): 1854-1861 (1992)) or Miller et al. (J. Bacteriology, 169(8): 3508-3514 (1987)).

Conclusion

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Richard G. Hutson whose telephone number is 571-272-0930. The examiner can normally be reached on M-F, 7:00-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Mondesi Robert can be reached on 571-272-0956. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

rgh
11/24/2010

/Richard G Hutson/
Primary Examiner, Art Unit 1652